07/01/16

Transformations didn't work maybe the temperature were too high.
I will look again these boxes tomorrow to know if I do it again.

07/04/16

No results on boxes for transformations

- Transformation plasmid IDT DSIP, NLP22

05/07/16

- Preparation for PCR WC1 , WC2, pRPS-27, pFRQ

[put pFRQ results]

I obtain the same amplification than the other, that is why next time I will use 2µL of DNA for pFRQ.

- **Transformation of plasmids DSIP, NLP22, pFRQ (2nd part)**
  - On a bacterial tube put 3ml of LB/ tube + 1,5 µL of AMP (x2000)
  - Touch the gel with bacteria with a cone and after soak it in your tube with LB
  - Put the tube on the Shaking Incubator

- **Préparation de gel d'agarose 1%**
  400 ML de TAE x0,5 + 4g d'agarose

07/06/16

- **PCR**

No result for pRPS-27 PCR, preparation of other tube (elongation time 30s)

Preparation of pFRQ with 2µL of DNA
PCR WC1 and WC2 made and migration for tomorrow

- **Transformation 3rd part (DNA extraction)**

Be careful, our DNA extraction kit is old, for the wash solution, you have to prepare it yourself.

For 3 tubes :
in a 15mL Tube : 800µL Wash solution + 2,2 mL EtOH 96%

Advice : For the elution step at the end , after you put the Elution solution , wait 5 min for a better elution (for more DNA)

Follow the extraction protocol on the kit with these advices

NB : In the fridge you can find 3 tubes with transformed bacteria and 3 boxes with bacteria transformed.

07/11/16

No more gDNA left. Sabrina put a “culture” a worms.

Replate DSIP, NLP22, P2A, pFRQ in 4ml LB , put in the incubator

Redo PCR of

- WC2 from gDNA of N. crassa : not enough PCR product. Then increase elongation time.
- pFRQ from plasmid *number* : not enough PCR product. Then increase elongation time.
- pUnc-119 : elongation and hybridation times have been inverted last time.
- pRPS-27 : elongation and hybridation times have been invertted last time.

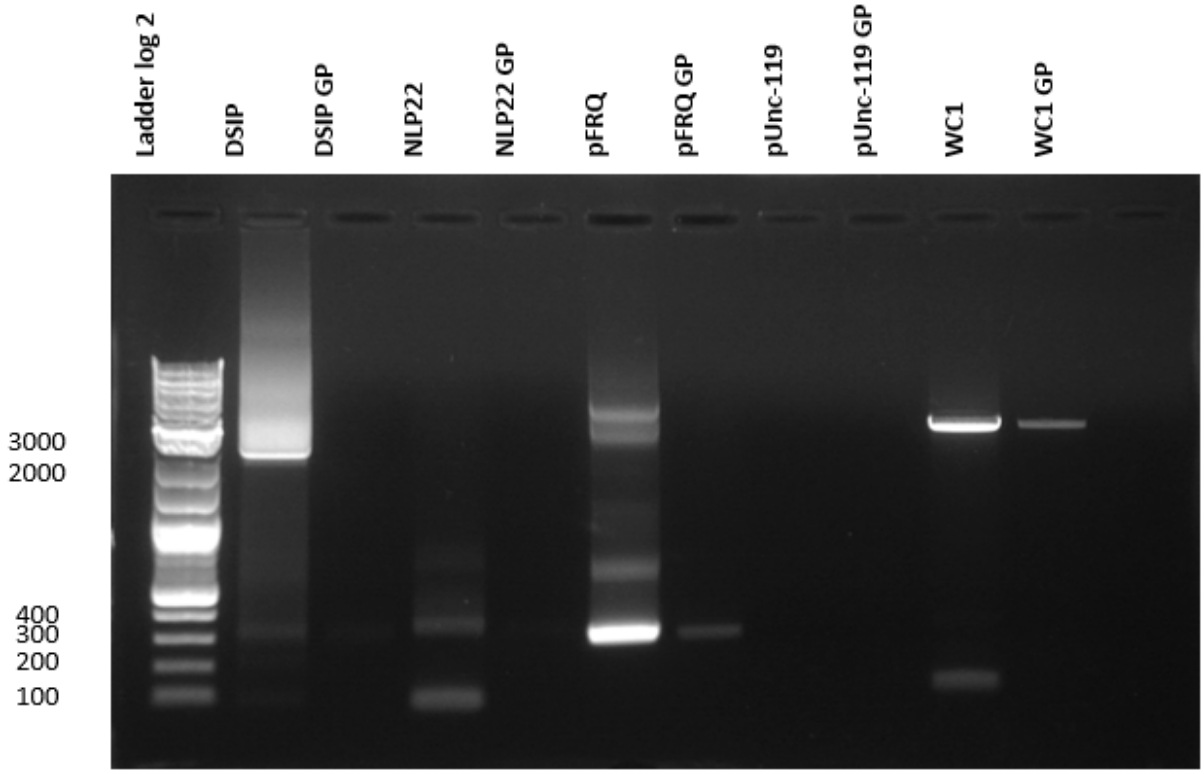
Resuspend GFP from plasmid (Table4) and RBS from plasmid (Table4).
Concentrations :
RBS : 110,8 ng/ul
GFP : 105,5 ng/ul

Try to double digest this DNA with EcoRI and SpeI. No DNA on 1% agarose gel.

Transformation of resuspended GFP and RBS (Table4) for stocks. Let grow overnight on chloramphenicol (as suggested by plasmid maps) on amp (as suggested by iGem parts).

07/12/16

migration of PCR of pFRQ, pUnc-119, pRBS-27 (evaporation… 8ul left) and WC2.
All were ok.
Gel purifications of PCR products on a 1% agarose gel.



Transformations gave many colonies on amp plates, zero on chloramphenicol plates.
Set up 5 mini cultures for dirty mini preps of GFP and RBS in 3ml of LB+amp. Let grow ⅚ hours at 37°C.

Prepare P2 and P3 solutions for dirty mini preps : Qiagen protocol.

07/13/2016

Dirty mini preps of GFP and RBS at . 500 ul of bacteria frozen into 15% of glycerol 100% for stocks.
Digest of DNAs with EcoR1 HF, PstI and DpnI in Master Mix (from iGem protocol http://parts.igem.org/Help:Protocols/Linearized_Plasmid_Backbones).
Set up starter cultures to set up maxi cultures the next day (we gorgot to keep 1 mL of bacteria to directly set up maxi cultures).

Digest of pSB1C3 from iGem and from amplification by Marguerite and Fatma with EcoR1 HF, PstI and DpnI (Master Mix). Gibson assembly with pFRQ GP or WC1 GP as insert :

	VI	V (linearized)
pSB1C3 pFRQ GP or WC1 GP Master Mix dw	2,5 2,5 5 _	2,5 _ 5 2,5

Oups, redo, but think of dephosphorylate the pSB1C3 before Gibson Assembly !!!!

Transformation of thermo competent bacteria and grow at room temperature (next day is off).

07/14/16

- Backbone transformation (pSB1C3)

20 uL competent bacteria + 1 uL DNA
20 min at 4°C
30 s at 42°C
5 min at 4°C

add 250 uL LB

1h at 37°C
Put in petri box with AMP
I made a mistake our backbone is Chlo resistant tomorrow I have to prepare it again.

- Test GFP and RBS

mettre le gel

We choose GFP and RBS 2 to put back in maxi culture.

- Gel purification NLP22

mettre

Great results , DNAs have been pooled in a single tube.

07/18/16

extraction ADN c.elegans
voir protocol kit lyse tissus
concentration : 92,5 ng/uL ratio 2,03

Transformation DSIP, nlp22 , pFRQ
cf potocole transformation .

remise en culture de pSB1C3 dans 4ml le matin et mise en culture dans 200ML LB + Chlo , overnight

Maxi preps of GFP (73,6 ng/ul, ratio 260/230 = 1,87) and RBS (63,4 ng/ul, ratio 260/230 = 1,76) .

07/20/16

Maxi prep of pSBIC3 and resuspension in 500 ul of water (353 ng/ul, R 260/280 = 1,92)
PCRs of pRPS-27, pxBP1, pUnc-119 and gel purifications :
pRBS-27 : 11,8 ng/ul (R 260/280 = 3,27)
PXBP1 : 68,6 ng/ul (R 260/280 = 2,23)
pUnc-119 : 47,7 ng/ul (R 260/280 = 47,7 ng/ul)

07/21/16

verif colonies precedentes : pas de pertes
verif efficacité antibio: Amp RAS

07/22/16

Transfo BBa_K1537016 (Peptide 2A)
resistance Chloro

07/23/16

Gibson reaction with these gel purified inserts :
DSIP, NLP22, pFRQ, pUnc-119, pXBP1, WC1, WC2

	Vector alone	Vector + insert
gel purified pSB1C3 (not dephosphorylated)		

Gibson reaction of gel purified pSB1C3